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EVIDENCE FOR REPRODUCTIVE ISOLATION BETWEEN *XESTIA ADELA* FRANCLEMONT AND *XESTIA DOLOSA* FRANCLEMONT (LEPIDOPTERA: NOCTUIDAE)

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Abstract.—Males of the sibling species *Xestia adela* Franclemont and *X. dolosa* Franclemont respond preferentially to conspecific virgin females in numbers that indicate the presence of specific differences in sex pheromones. No fertile eggs were obtained from laboratory crosses between the species, although some mating occurred.

The results of pheromone studies within the species complex that includes the Eurasian *Xestia c-nigrum* (L.) and the North American *Xestia adela* Franclemont and *Xestia dolosa* Franclemont require some further investigation when considered with relationships proposed on the basis of morphological and genetic characters (Franclemont, 1980; Hudson and Lefkovitch, 1982).

Bestman et al. (1979) have shown that *X. c-nigrum* (as *Amathes c-nigrum*) in the vicinity of Frankfurt, West Germany are attracted to cis-7-tetradecenyl acetate. Similarly in Hokkaido, Japan, Fujimara (1976) and Hirai (1976) found that cis-7-TDA attracted males of *X. c-nigrum* (also as *A. c-nigrum*), whereas the trans isomer did not. Subsequently, Hirai (1976) extracted material from 2000 virgin females and found that the active factor eliciting responses from antennal receptors was indeed cis-7-TDA. North American representatives of the species, formerly known as large and small forms of *A. c-nigrum*, were found by Roelofs and Comeau (1971) to be differentially attracted to the two isomers, the large form (now *X. dolosa*) to cis-7-TDA and the small form (now *X. adela*) to trans-7-TDA. Further trials did not confirm these results, and it is possible that at this time some contaminants were present in the synthetic attractants. European *X. c-nigrum* and North American *X. adela* have been shown to have more characters in common than *X. adela* and *X. dolosa* (Franclemont, 1980; Hudson, 1981; Hudson and Lefkovitch, 1982); specimens of *A. c-nigrum* obtained from Osaka, Japan also appear to be morphologically and genetically closer

to *X. adela* (Hudson, unpublished data), so that such interchange of behaviour towards the isomers of 7-TDA would be unexpected.

The purpose of the present study was to determine if the sex attractants for *X. adela* and *X. dolosa* are different by observing the attraction of males from wild populations to traps baited with virgin females, and to examine the possibility of interbreeding between laboratory colonies of the two species.

Material and Methods

The moths used in field and laboratory experiments were second and third generation insects reared from founder females obtained from North Gower (NG) and Harrow (HA), Ontario. Several lines of each species were crossed in the second generation. The moths were maintained in an incubator at 75°F and approximately 70% RH, with a photoperiod of 16 hours. Larvae were reared on an artificial diet as described by Hinks and Byers (1976).

In pheromone experiments Pherocon 1C (Zoecon) sticky traps were set up near Mallorytown, Ontario in an area close to the St. Lawrence Seaway where both *X. adela* and *X. dolosa* are known to occur. Twenty traps (8 baited with *X. adela* females; 8 with *X. dolosa* females; and 4 blanks) were maintained during the period August 28 to September 24, 1980, when the second brood of the wild populations were flying. Sixteen traps, including the 4 blanks, were attached at a height of approximately 1 m along a fence separating fields of oats and buckwheat from a wooded area; the remainder were hung from branches of small trees in the wooded area. The positions of the traps were randomized. Baited traps contained two 3-day old virgin females, each individually housed in a plastic mesh cage. The females were replaced every third or fourth day and the trapped males were identified by electrophoresis (Hudson and Lefkovitch, 1980) and by dissection (Hudson, 1981); sample collections have been retained in the Canadian National Collection of insects in Ottawa.

Mating experiments were carried out in wooden frame, plastic-mesh cages 30 × 30 × 17 cm in a rearing room maintained at 70°F and 70% RH with a 16 hour photoperiod. Five males and five females were used in all combinations, they were put into the cages immediately following eclosion until a substantial number of eggs had been laid in the control cage, then females were examined for spermatophores and in most cases for the presence of sperm in the spermatheca.

An attractancy index (A) was calculated substituting A for I (isolation index) in the formulae of Wasserman and Koepfer (1977):

$$A = \frac{\text{No. conspecific } \delta\delta \text{ attracted (CS)} - \text{No. other species } \delta\delta \text{ attracted (OS)}}{\text{Total no. } \delta\delta \text{ (both species) attracted (CS + OS)}}.$$

Table 1. Observed numbers of males caught in female-baited traps at Mallorytown, August–September, 1980. Expected numbers in parentheses.

	♀ Bait				
♂ Caught	*X. adela*	*X. dolosa*	Blank	Total	
X. adela	741 (660.9)	103 (183.1)	0	844	
X. dolosa	6 (86.1)	104 (23.9)	0	110	
Total	747	207	0	954	$\chi^2_1 = 388.4$
Attractancy index (A)	0.76 ± 0.022	0.89 ± 0.044			

The standard error was calculated as

$$SE = \left[\frac{1 - A^2}{CS + OS} \right]^{\frac{1}{2}}.$$

A value of $A > 2 \times$ standard error is considered to indicate significant conspecific attraction. A positive index indicates a preference by males for conspecific females, a negative index that no preference is shown.

Results

Attraction of field population males to virgin females.—The number of males caught in traps baited with virgin females of each species (Table 1) indicates significant conspecific attraction (chi-square = 388.4). Expressed as an attractancy index (A) and standard error (SE), from the formulae described in the previous section, for *X. adela* A = 0.76 ± 0.022 and for *X. dolosa* A = 0.89 ± 0.044; these are significant ($2 \times SE < A$).

The number of *X. adela* males collected remained fairly constant over the total time period, for example 18.0% (153) were collected on August 28 and 18.7% (158) on September 16. The total number of *X. dolosa* males collected was lower, but the numbers increased towards the end of the period, 10.5% (11) were collected on August 28 and 42% (44) on September 16. Traps hung along the fence bordering the oat field collected 80% (678) of all *X. adela* males and 72% (80) of all *X. dolosa* males, the remainder were collected in the tree traps.

Interspecific breeding experiments.—*Xestia adela* adapts very easily to laboratory rearing and 83% of the females in control cages were found to contain from 1–4 spermatophores. Mating success was unaffected by crossing NG and HA populations. *Xestia dolosa* intraspecific crosses were less successful and only 38% of the females contained spermatophores at the time of dissection. Interpopulation crosses beween *X. dolosa* males and females produced infertile eggs only, but only two trials were made because of a scarcity of *X. dolosa* NG at that time.

Table 2. Crossing experiments between *X. adela* and *X. dolosa*.

Species combination ♂ × ♀	No. Trials	Fertile Eggs	Infertile Eggs	Max. No. Spermatophores per Female	% No. Females that had Spermatophores
adela × *adela*	10	++[1]	+	4	85
dolosa × *dolosa*	14	+	++	2	38
adela × *dolosa*	12	−	+	3	15
dolosa × *adela*	11	−	++	1	5

[1] ++ = over 200 eggs/♀; + = less than 200 eggs/♀; − = no eggs.

When *X. adela* males were caged with *X. dolosa* females, spermatophores were deposited and sperm were found in the spermatheca of one female, but no fertile eggs were laid (Table 2). Multiple mating was demonstrated by one female that had three spermatophores. In this combination 15% of the total number of females were found to have spermatophores either completely or partially deposited (with part extruding from the ductus bursae). Several pairs were observed in copula and three of these were unable to separate.

In crosses between *X. dolosa* males and *X. adela* females only 5% (2) of the total number of females (55) had one spermatophore each. There was no evidence of sperm in the spermathecae of these females and no fertile eggs were obtained, although many infertile eggs were laid.

Discussion

The results of attractancy tests using virgin females strongly indicate that the sex pheromones of *X. adela* and *X. dolosa* are different, but that some cross attractancy does occur. The highest proportion of cross attractancy was between *X. adela* males and *X. dolosa* females, but some also occurred in the reciprocal combination, particularly towards the end of the collecting period when there were more *X. dolosa* males flying.

No fertile eggs were obtained from interspecific no-choice crosses set up in the laboratory. The evidence that mating occurred between both combinations of males and females and yet only infertile eggs were produced indicates that circumvention of the long-distance (anemotactic) responses (Roelofs and Cardé, 1977) did not induce hybridization, and it probably means that both premating and post-mating isolation mechanisms are acting together to maintain the taxonomic discreteness of the species.

A measure of genetic differentiation between *X. adela* and *X. dolosa* and an European population of *X. c-nigrum* was obtained from an electrophoretic study of allozyme frequencies (Hudson and Lefkovitch, 1982). Distances between the species were calculated from the formulae of Nei (1972) as genetic distance (D) and suggested a closer relationship between *X. c-*

nigrum and *X. adela* (D = 0.104) and between *X. c-nigrum* and *X. dolosa* (D = 0.260) than between *X. adela* and *X. dolosa* (D = 0.319). These relationships could be interpreted as the result of two separate introductions of *X. c-nigrum*, one of which, as *X. dolosa*, remained restricted to the eastern and central regions of N. America, and the other, as *X. adela*, became distributed transcontinentally. Where the two species are sympatric reproductive isolation has been achieved in part by pheromone differentiation, possibly brought about by the presence of different proportions of attractant components; this could explain the cross-attractancy observed in our experiment.

The larger number of *X. adela* males attracted to *X. dolosa* females could be interpreted as a one-sided mating preference, but is more likely to be due to the larger number of *X. adela* males in the field population during the observation period.

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